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Responses of an Estuarine Population of the Blue Mussel 'Mytilus edulis' to Heated Water from a Steam Generating Plant

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Responses of an Estuarine Population of the Blue Mussel *Mytilus edulis* to Heated Water from a Steam Generating Plant*

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Abstract

An entire bed of the blue mussel *Mytilus edulis*, consisting of 5,000 individuals/m^2, died during June, 1971 in the effluent canal of a steam generating plant when the temperature increased above 27°C. Similarly, the population in the intake canal disappeared when temperatures rose above 27°C in August. Laboratory studies showed that *M. edulis* could not tolerate continuous temperatures above 27°C, and feeding stopped shortly after the mussels were exposed to 25°C. Histopathological studies indicated that the cause of death of this bivalve was associated with degeneration of the frontal and laterofrontal cilia of the columnar epithelium of the gill filaments. In conjunction with this, there was necrosis and sloughing of the epithelium of the intestinal diverticula. Extensive amoebocytic infiltration was noted in the byssogenous cavity, gill filaments and stomach wall.

Introduction

This report is part of an extensive study undertaken of the intake, discharge and adjacent waters of the steam generating plant operated by New England Electric Company in Somerset, Massachusetts, USA, at Brayton Point from March, 1970 to September, 1972. Most of the research was directed to the study of effects of heat on zooplankton and the benthic amphipod *Corophium isidiosum*. However, during mid-summer 1970, a piece of cyclone fence retrieved from the bottom of the discharge canal bore a large population of dead *Mytilus edulis*. This fact and the assumption that the kill was caused by high temperatures in the discharge canal led us to attempt a more comprehensive study of this mollusc.

The research program was divided into field and laboratory studies. Growth of the mussels and their response to the increasing temperatures in the effluent canal was followed from the time spat were first observed until the population died. Laboratory studies consisted of determination of the highest constant temperatures mussels could withstand and the effect of increasing temperatures on feeding rates. Current techniques in histopathology were used to determine the magnitude of the lesions induced by the high temperatures.

*This paper represents part of a dissertation by the first author, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Rhode Island, January 1973.

Materials and Methods

Field Studies

Growth of the mussel *Mytilus edulis* was measured from the time the spat was first observed in March, 1971, until the population died in June, 1971. Random scrapings containing mussels were obtained from rocks or any other substrate and pooled. Subsequently, all individuals were separated from debris and preserved in 50% ethyl alcohol. Within 3 days of collection measurements were made with a vernier caliper accurate to ±0.003 cm. Only mussels from the intake and effluent were used for this study on the assumption that, except for heat stress at the effluent canal, other hydrographic conditions were similar.

Several live specimens were collected at the effluent canal in early June, 1971, when the temperature was 25°C. In

mid-June, when temperatures were between 28° and 30°C and 50% of the *Mytilus edulis* had died, another group of mussels was sampled for histopathological studies.

The mussels were fixed for 24 h in Zenker-formol solution made with zinc chloride (McClung and Allen, 1964). After fixing, the tissue was washed in tap water overnight and transferred to 70% ethanol. Standard histological techniques of dehydrating, clearing, embedding, and sectioning were then carried out. Tissue sections 6 µ thick were stained with Harris hematoxylin eosin.

Laboratory Experiments

Laboratory experiments on *Mytilus edulis* were conducted in two different ways. First, 100 specimens were taken at random in Narragansett Bay during February, 1972, and divided into 4 groups of 25 individuals. Two groups were held as controls at 3°C. The other two groups of 25 mussels each were placed in a temperature-controlled bath, and its temperature raised stepwise at a mean rate of 1.1C°/day (range 0.5° to 2°C). This rate was chosen to avoid any possible shock reaction by the experimentals. Control and experimental mussels were fed every 2 days with 8 l of a *Monochrysis lutheri* culture containing over two million cells/ml, grown in enriched seawater (Guillard and Ryther, 1962). Cell counts of the food organism were made immediately after feeding, and 8 to 12 h later with an electronic counter. When temperatures in the laboratory were raised above 27°C, *Mytilus edulis* valves remained open most of the time, and would close slowly only when the mantle was stimulated. At this stage, some mussels were fixed for histopathological examination as previously described.

A second experiment was designed to investigate the feeding efficiency of *Mytilus edulis* after prolonged exposure to 25°, 26°, 27° and 28°C. Further attempts were made to determine the histopathological changes induced by these long exposures.

In March, 1972, 120 mussels were collected from Narragansett Bay. Twenty were held in each of two aquaria (A and B) at 2.5°C, the water temperature at the time of sampling. The remaining mussels were divided in groups of 20 and held in aquaria (C, D, E, and F) in which the temperature was raised at a rate of approximately 1C°/day (range 0.6° to 1.9°C) until 25°C was attained. This part of the method constitutes Part I of Table 2 and lasted for 33 days. At this point, one aquarium (C) was maintained at 25°C and D, E, and F were raised to 26°, 27° and 28°C, respectively. Table 2 depicts this portion of the experiment as Part II and was carried out for 5 days. All mussels were fed daily with a culture of *Monochrysis lutheri* containing over 2 million cells/ml; aquaria were aerated continuously. Cell counts of the food were made immediately after feeding, every half hour thereafter for 2 h, and a final count 12 h after the initial feeding. Control mussels almost completely cleared the water of food cells by the end of this time.

Results

Field Work

Field observations made in the intake and discharge canals showed that the population density of *Mytilus edulis* was very high in the effluent canal, where 5,000 mussels/m^2 were counted at the mean low tide mark in March, 1971 and approximately 20,000 mussels/m^2 in May, 1972. On both occasions no mussels appeared to settle in the intake area until May. Fig. 1 summarizes the growth of the molluscs from March to June, 1971. Fast growth in the effluent was evident from the slope of the curves. In general, growth was 2 mm/week in effluent waters and 0.82 mm/week in intake waters. The first spat attained sexual maturity and spawned by early June; Fig. 2A and B depict gonadal development of females and males, respectively, showing indications of spawning at this time.

By June 18, 52% of the population died when temperatures between 28° and 30°C prevailed throughout the 24-h period in the effluent canal. Two days later, the entire population of mussels in the effluent area was dead, the mussels detached, and all empty shells washed out of the canal. The mussels that set in May in the intake area continued growing normally, but died by the end of the summer. Temperature had risen above 27°C by the time of their death (Fig. 1).

Further studies were made in June, 1972 in an attempt to assess the predictability of the death of *Mytilus edulis* in relation to the discharge of heated water at the same steam generating plant. Early in June a temperature of 27.5°C was recorded during the morning hours, yet the population of mussels was intact but showing signs of stress. A continuous temperature recorder was placed *in situ* to follow the temperature variations during a 24-h period. Table 1

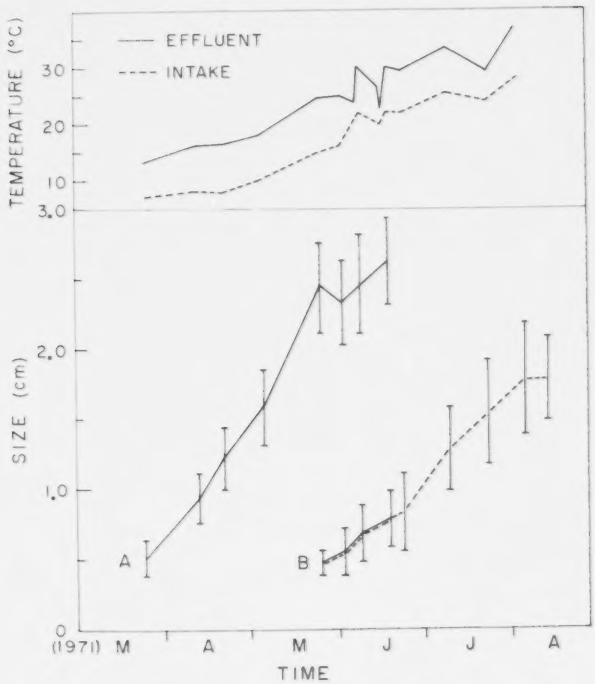

Fig. 1. Upper graph: intake and effluent temperature from March to August, 1971. Bottom graph: growth of *Mytilus edulis* in (A) discharge canal of Brayton Point power plant from March to June (fall spat); (B) same discharge canal (solid line) from May to June (spring spat) and in intake canal (dashed line) from May to August (spring spat)

shows that at the beginning of the month, temperatures were high in the daytime, but decreased during late night hours.

Frequent sampling followed by counts of dead and live *Mytilus edulis* in each sample indicated that mussels started to die as the temperature was raised above 28°C, but mortality was low (about 4% of the total). However, the rate of mortality increased as temperatures of 27°C and above prevailed during the 24-h period, particularly after the middle of June. To our surprise in June, 1972, 50% of the population of *M. edulis* was dead on exactly the same date as reported for 1971 (June 18).

Histopathological studies of *Mytilus edulis* from the discharge canal indicate that mussels thus exposed show degeneration of the frontal and laterofrontal cilia of the gills (Fig. 3B), sloughing of the intestinal diverticula, and considerable amoebocytic infiltration in the stomach, gills, food grooves and byssogenous cavity. Mussels from the intake showed no signs of gill damage (Fig. 3A) or of any other organs.

Laboratory Experiments

Fig. 4 shows percent mortality (of the total) of *Mytilus edulis* as the temperature was raised by a mean of 1.1C° each day. No mussels died below 26°C, although some were slowly closing their

Table 1. Hourly temperatures (°C) in effluent canal during half of the first part of June, 1972. -: No data

Time (hrs)	June 1	June 2	June 3	June 4	June 5	June 12	June 13	June 15
00.30	-	28.0	27.8	30.0	27.0	-	28.2	28.5
01.30	-	26.3	27.0	29.5	25.0	-	27.2	28.8
02.30	-	27.0	26.8	28.0	24.5	-	26.8	28.0
03.30	-	29.0	27.8	26.5	24.7	-	26.0	28.5
04.30	-	25.8	25.0	25.5	24.8	-	26.2	29.0
05.30	-	29.0	24.0	25.0	-	-	26.5	29.2
06.30	-	25.1	24.5	25.0	-	-	27.4	29.2
07.30	-	28.0	25.0	25.0	-	-	29.0	30.0
08.30	-	28.8	27.5	25.8	-	-	28.8	30.0
09.30	-	28.0	28.2	27.3	-	-	28.4	30.0
10.30	27.5	28.8	28.0	29.0	-	28.0	28.0	29.5
11.30	-	-	28.0	29.0	-	28.5	28.5	29.5
12.30	29.1	28.5	28.2	29.5	-	28.2	28.8	29.8
13.30	29.0	29.5	28.5	29.7	-	28.0	28.8	29.8
14.30	28.0	30.0	29.5	30.3	-	28.5	29.0	30.0
15.30	28.5	29.5	29.5	31.4	-	28.2	29.0	29.8
16.30	28.8	29.5	29.3	31.8	-	28.0	29.0	30.0
17.30	28.5	29.5	29.5	31.0	-	28.0	29.0	30.0
18.30	28.3	29.5	30.0	31.0	-	28.5	29.2	31.0
19.30	28.5	29.5	29.8	31.5	-	28.5	29.0	31.5
20.30	28.5	29.2	29.2	31.0	-	28.3	28.5	30.0
21.30	28.5	29.0	29.9	29.5	-	28.2	28.2	30.5
22.30	28.5	29.0	30.3	28.5	-	28.0	28.2	30.3
23.30	28.0	29.0	30.5	28.0	-	28.0	28.9	29.5

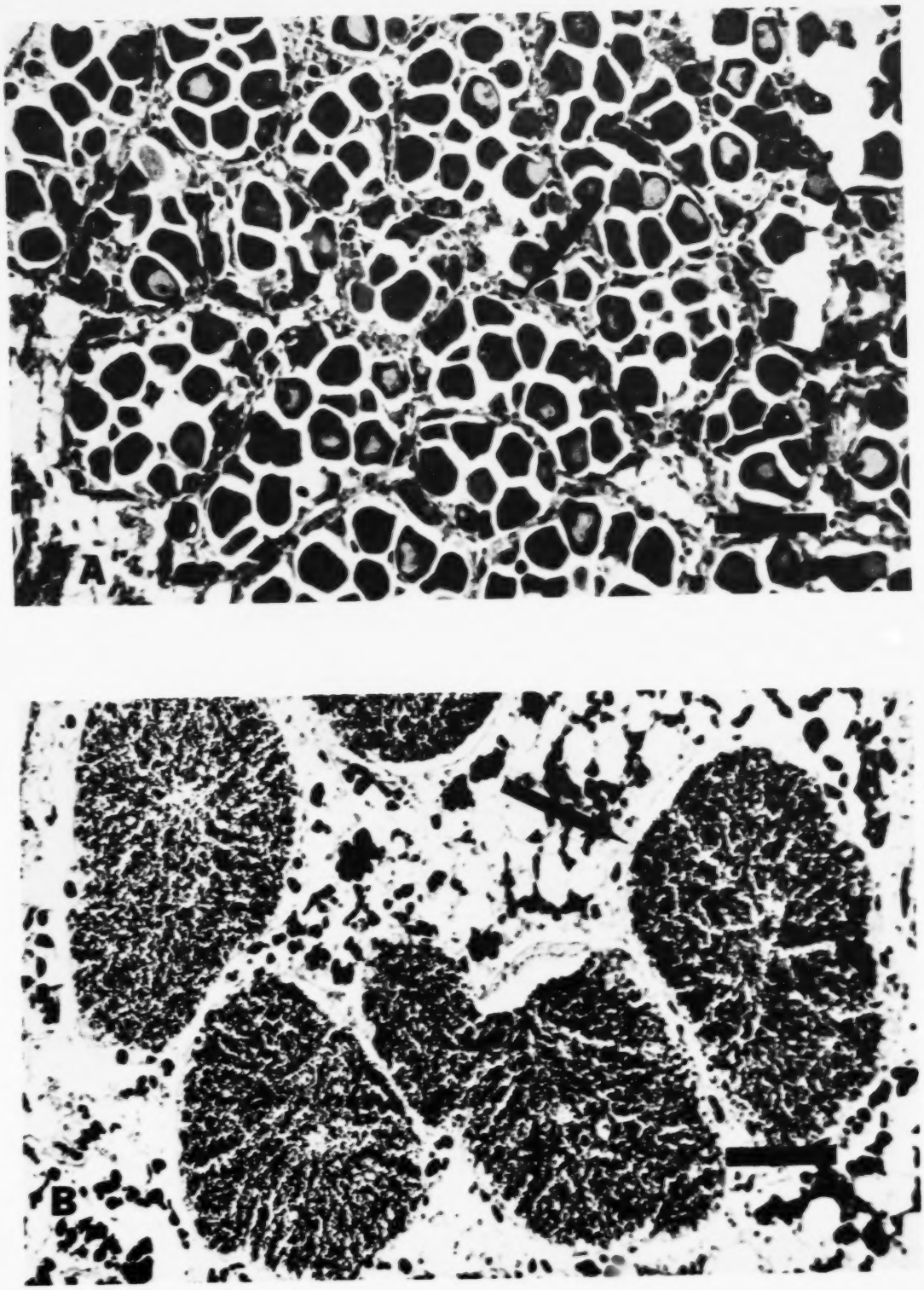

Fig. 2. *Mytilus edulis*. Cross sections of (A) female reproductive tract (arrowed) showing various stages of development of the ovae; (B) male reproductive tract (arrowed) showing follicles filled with spermatozoa. Hematoxylin and eosin. (Scale bars = 100 μ). Mussels were taken from discharge canal in June, before temperature rose above 26°C

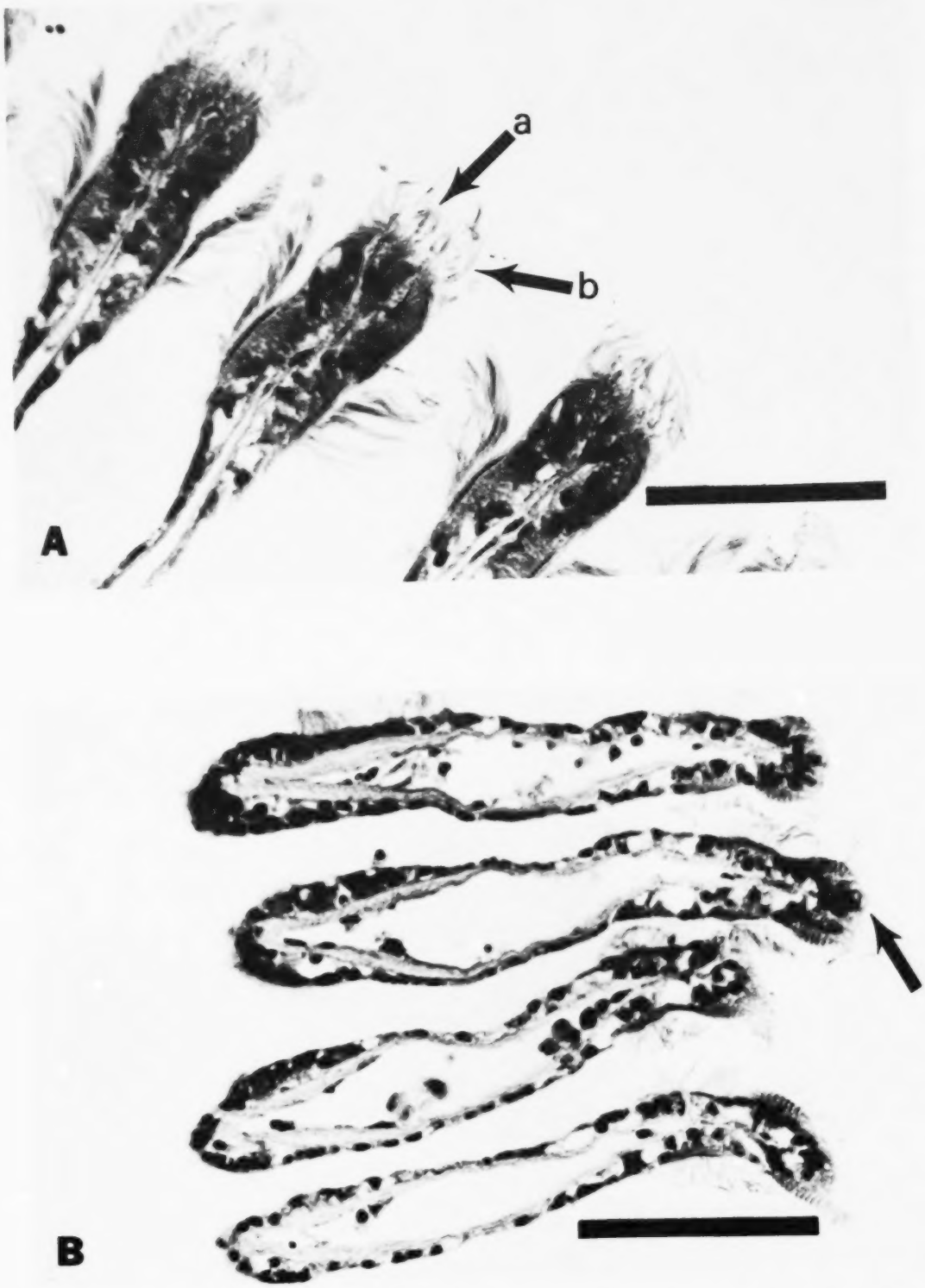

Fig. 3. *Mytilus edulis*. Sagittal sections of gill filaments of (A) control specimen from intake, showing normal epithelium and frontal (arrow a) and laterofrontal (arrow b) cilia; (B) specimen from effluent canal at 30°C; note loss of frontal cilia (arrow). Hematoxylin and eosin. (Scale bars = 100 μ)

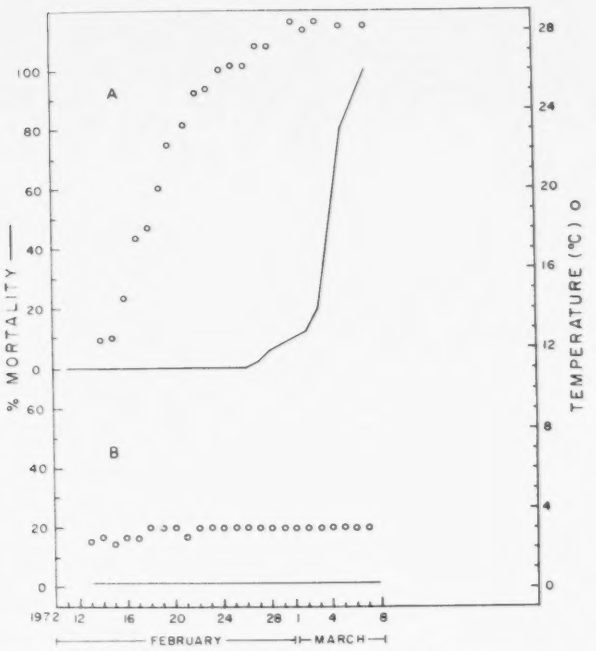

Fig. 4. *Mytilus edulis*. Percent mortality of total mussels during mean rise in temperature of 1.1C°; (A) exposed, (B) control mussels. Circles refer to temperature on right axis; line represents mortality on left axis

shells at 26°C. The initial records of mortality, 2 and 6%, occurred after the first and second day, respectively, at 27°C. As the temperature was raised to 28°C the mortality increased to 10%, but signs of stress were more severe. At this point, the mussels kept their valves open and would close them slowly only after being poked lightly with the tip of a pipette. Once mussels attained this stage and the temperature was held at 28°C, mortality increased rapidly. No mussels died in the control group during the study.

The percent of food utilized during this period also appeared to be related to temperature stress. From the start of the experiment until a temperature of 24°C was attained, the mussels utilized 99.9% of the food dispensed in the tanks within 12 h. At 25°C, there was a small decrease to 96.5%, but at 28°C food consumption was only 33%. It would seem probable that, with a decrease in the number of mussels, there would be an excess of available food. However, this assumption proved to be untenable because it was found that as few as 3 healthy specimens would consume, in 12 h, 78% of the food dispensed in their tank if held at a temperature of 15°C.

In the second experiment, no mortalities were recorded when the temperature reached 25°C. Experimental and control mussels ate all the food within 8 to 12 h after feeding. Table 2 summarizes the results. It is evident from these data that the feeding of experimental mussels decreases during exposure to continuous temperatures of 25°C and above, the condition worsening with time and temperature increase.

Histopathological examination of sagittal sections of laboratory-exposed *Mytilus edulis* revealed tissue changes in the gills, byssus gland and stomach. The gill filaments of the exposed mussels showed a loss of the frontal and laterofrontal cilia of the ciliated columnar epithelium and loss of their cytoplasm (Fig. 5B). This condition contrasts markedly with the gill filaments of a control specimen illustrated in Fig. 5A.

The number of mussels with loss of cilia increased as the temperature increased, from 75% at 25°C to 94% at 26° to 28°C. Loss of cilia was also noted in the ciliated columnar cells of the food groove; however, the cytoplasm in these cells was normal.

Amoebocytic infiltration of the epithelium (Fig. 5) illustrates a predominant feature of *Mytilus edulis* held at high temperatures. Some of the gill filaments were distended with accumulation of amoebocytes. The accumulation was so extensive at times that there was a complete distortion of the normal anatomy of the gill filaments in some specimens (Fig. 6).

At 26° to 28°C, the byssus gland showed necrosis and amoebocytic infiltration at the septa of the byssogenous cavity (Fig. 7B, exposed specimen; Fig. 7A, control). This condition was noted in at least 50% of the mussels.

At 26°C, foci of the stomach wall showed an infiltration of the mucosa by amoebocytes while the submucosa was completely infiltrated by the same cells. At 27° to 28°C, the infiltration was so extensive that large portions of the stomach were completely ulcerated and there was no indication of any normal ciliated columnar epithelium (Fig. 8B, exposed specimen; Fig. 8A, control). The intestine also showed amoebocytic infiltration of the intestinal wall; however, it was not as extensive as that seen in the stomach wall.

Discussion and Conclusions

It is evident from the information obtained that *Mytilus edulis* cannot survive temperatures above 27°C over extended

Table 2. *Mytilus edulis*. Effect of temperature on feeding activity. For further details see text

	Control tanks						Experimental tanks											
	A and B			C			D			E			F					
Part I 33 days	Mean temperature: 2.5°C. Mean uptake of food in 4 h: 99%. Condition of mussels: unstressed.			Temperature: raised from 2.5° to 25°C at a mean rate of 1.9°C (range 0.6°–1.9°C) every 3 days. Stress: none. Food utilization: mean of 99% in 4 h.			Temperature: same conditions as in Tank C. Stress: none. Food utilization: mean of 99% in 4 h.			Temperature: same conditions as in Tank C. Stress: none. Food utilization: mean of 99% in 4 h.			Temperature: Same conditions as in Tank C. Stress: none. Food utilization: mean of 99% in 4 h.					
Part II	Condition of mussels	Temp. (°C)	% food ingested	Condition of mussels	Temp. (°C)	% food ingested	Condition of mussels	Temp. (°C)	% food ingested	Condition of mussels	Temp. (°C)	% food ingested	Condition of mussels	Temp. (°C)	% food ingested			
Day 1	Unstressed	2.5	½h 50.5 1h 67.5 1½h 81.0 2h 88.5 12h 99.9	Stress not evident	25	½h 1 1h 5 1½h 25 2h 31 12h 94	Stress not evident	26	½h 10 1h 10 1½h 45 2h 29 12h 93	Stressed (10%)	27	½h – 1h – 1½h 4 2h 8 12h 82	Stressed (20%)	28	½h – 1h – 1½h – 2h 5 12h 80			
Day 2	Unstressed	2.5	½h 54.5 1h 84.5 1½h – 2h 85.5 12h 99.9	Stressed (10%)	25	½h 23 1h 38 1½h – 2h 56 12h –	Stressed (25%)	26	½h 20 1h 30 1½h – 2h 35 12h –	Stressed (55%)	27	½h – 1h 8 1½h – 2h – 12h –	Stressed (100%)	28	½h 3 1h 8 1½h – 2h – 12h 7			
Day 3	Unstressed	2.5	½h 60.0 1h 71.0 1½h 82.0 2h 86.0 12h 99.9	Stressed (25%)	25	½h – 1h – 1½h – 2h – 12h –	Stressed (55%)	26	½h – 1h – 1½h – 2h – 12h –	Stressed (100%)[a]	27	½h – 1h 16 1½h – 2h 16 12h 16	Stressed (100%)[a]	28	½h 10 1h 12 1½h – 2h 19 12h 19			
Day 4	Unstressed[a]		½h 50.0 1h 61.0 1½h 83.5 2h	Stressed (40%)[a]	25		Stressed (100%)[a]	26										
Day 5				Stressed (80%)[a]	25													

[a]Mussels were removed for Zenkers' fixation.

periods of time. These results substantiate what has been reported in the literature. Dodgson (1928) pointed out that the highest temperature at which mussels have been observed under natural conditions is 26°C. Wells and Gray (1960) noticed that *M. edulis* larvae are swept around Cape Hatteras during fall storms and settle in Beaufort, North Carolina. There they grow fast, attaining a size of 3 cm by the following June. However, the mussel disappears from these waters as soon as the temperature reaches around 26.7°C. They concluded that temperatures above 26.7°C are the cause of their mortality. Ritchie (1927) found that *M. edulis* from the British Isles were killed by an exposure of 14 h to a temperature of 28.9°C. Hutchins (1947), in his work on temperature as a factor in determining the geographical limits of species, suggested that the southern limit of *M. edulis* is 80°F (26.6°C); the maximum monthly mean (summer) surface temperature off Cape Hatteras, USA. Read and Cumming (1967) studied the mortality of the mussel and other molluscs with the passage of time at different temperatures. They found that time to 50% mortality was about 3 days for mussels held at 27°C and less than 2 days for mussels held at 28°C. A group exposed to 26°C sustained a 59% mortality after approximately 13 days exposure. Read and Cumming (1967) concluded that the distribution of *M. edulis* in the sublittoral zone is limited by sea temperatures in the neighborhood of 26.7° to 28.9°C.

Brenko and Calabrese (1969) studied combined salinity-temperature effects on larvae of *Mytilus edulis* reared in the laboratory and reported that survival was uniformly good, 70% or better, at temperatures between 5° and 20°C and salinities between 15 and 40‰. Optimum growth was observed at 20°C and salinities from 25 to 30‰; however, growth decreased at 25° and 10°C. Their results may explain why we did not observe any spat in the intake canal during the early spring months when the temperature ranged between 5° and 15°C.

Schulte (1975) determined the influence of algal concentration and tempera-

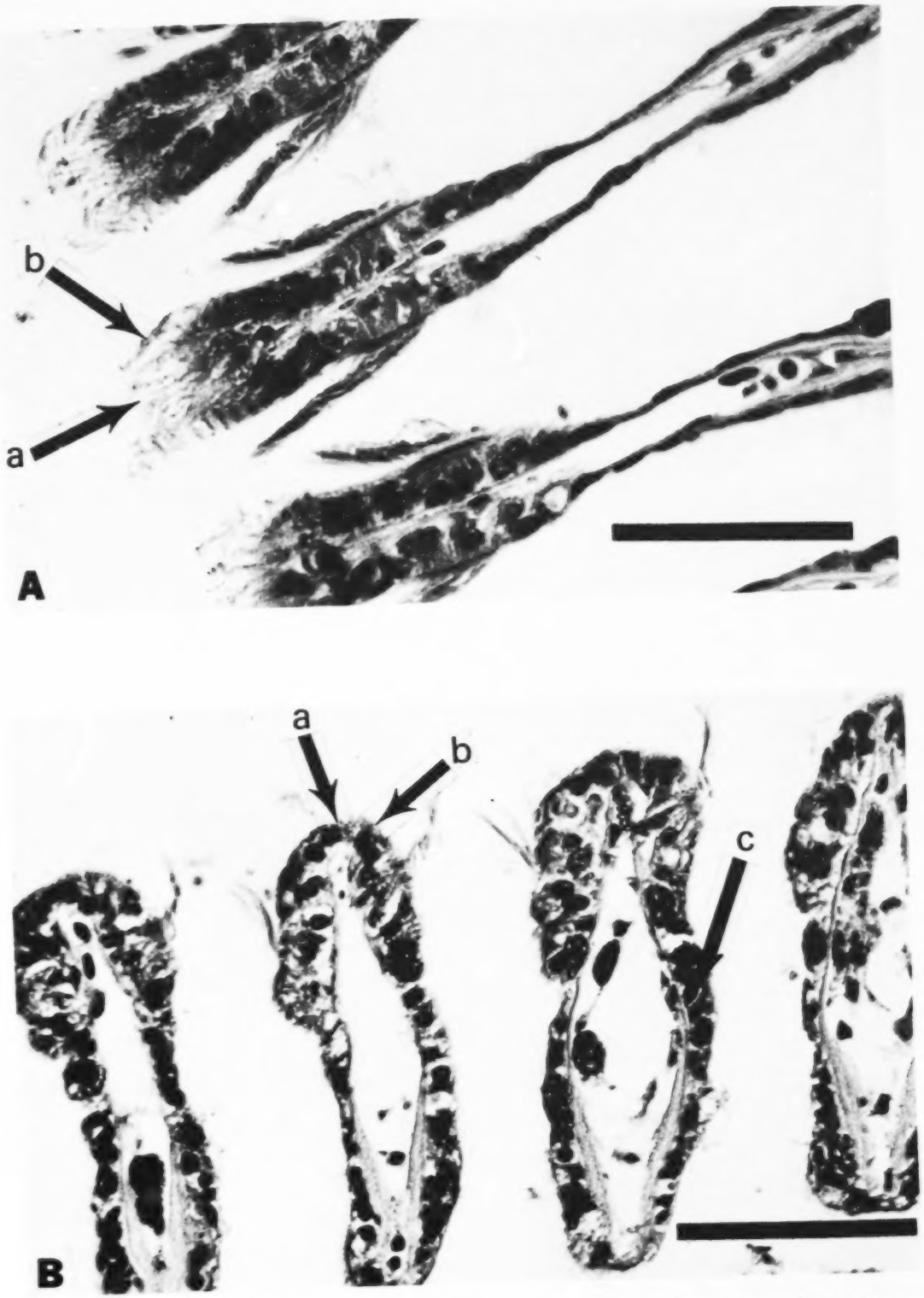

Fig. 5. *Mytilus edulis*. Sagittal sections of gill filaments of (A) control mussel showing normal epithelium and frontal (arrow a) and lateral (arrow b) cilia; (B) mussel exposed to 26°C — note loss of frontal and laterofrontal cilia (arrows a and b, respectively) and loss of cytoplasm from columnar cells (arrow c). Hematoxylin and eosin. (Scale bars = 100 μ)

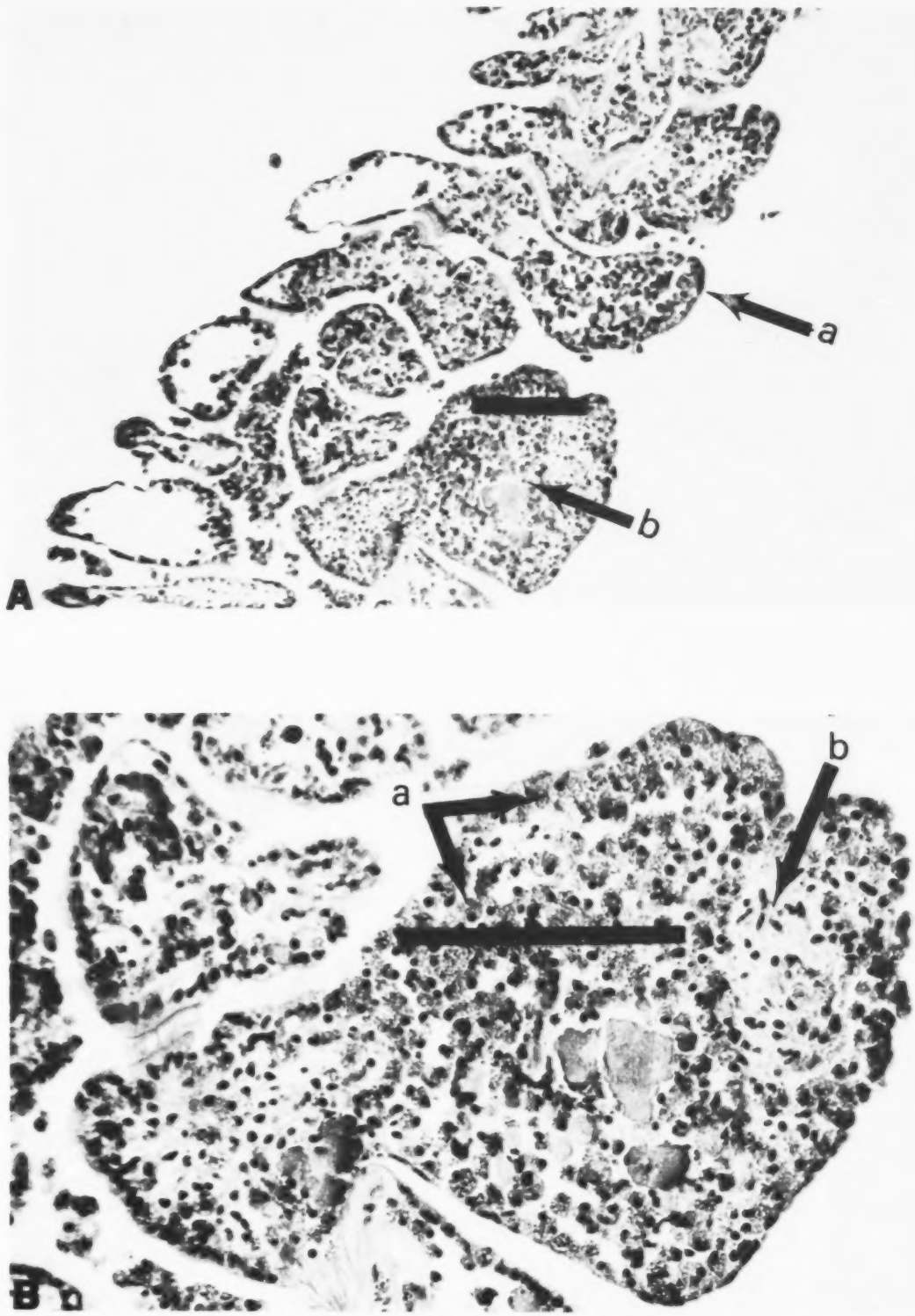

Fig. 6. *Mytilus edulis*. (A) Sagittal section of gill filaments of mussel exposed to 27°C showing extensive accumulation of amoebocytes in branchial vessels; note loss of cytoplasm and cilia (arrow a), and distortion of filaments; arrow b indicates area magnified in (B). (B) Higher magnification of area "b" in (A), showing accumulation of amoebocytes (arrow a) and cell debris (arrow b) in branchial vessel. Hematoxylin and eosin (Scale bars = 100 μ)

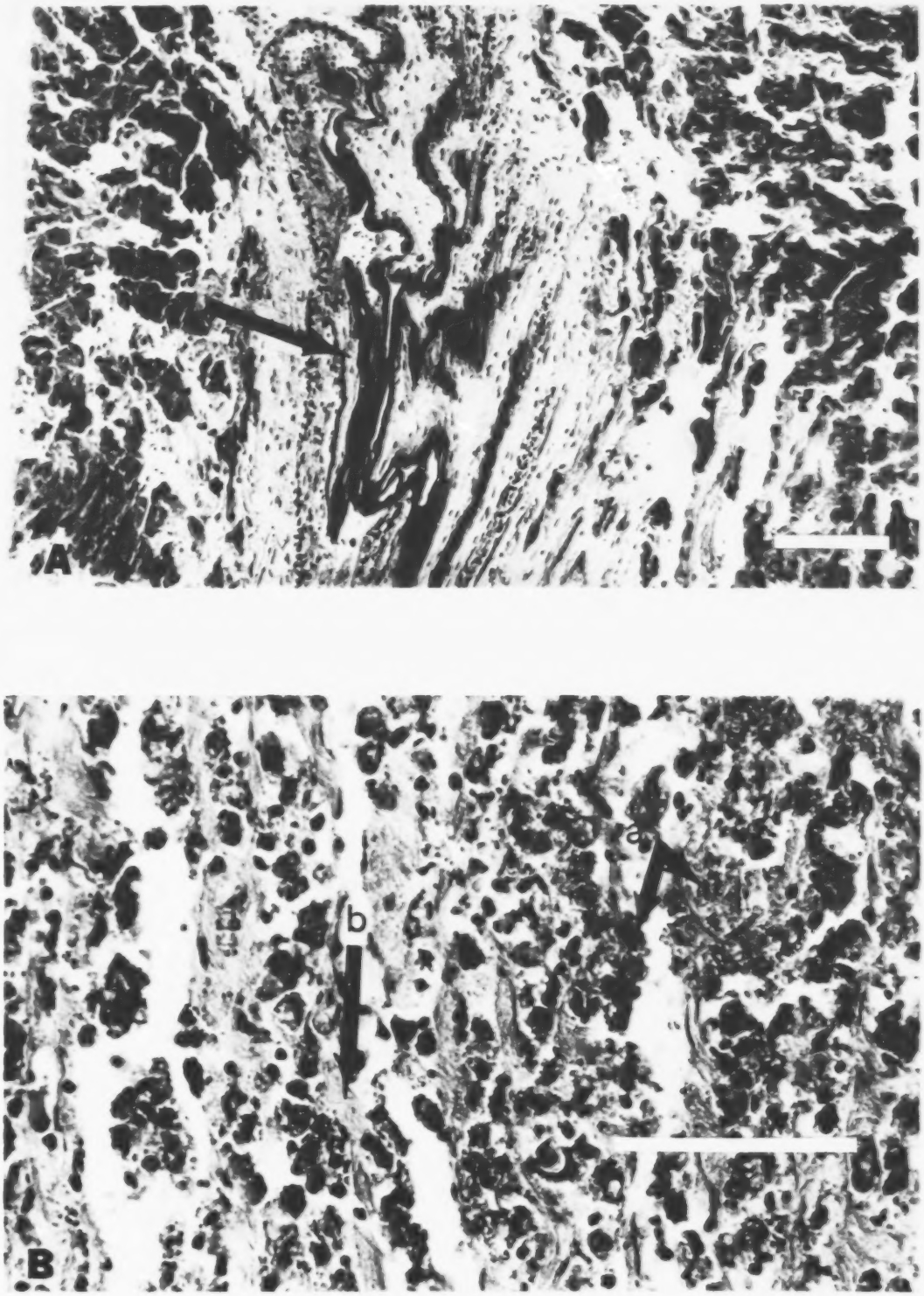

Fig. 7. *Mytilus edulis*. Microscopic section of byssogenous cavity (arrowed) of (A) normal control specimen; note septae (scale bar = 100 μ); (B) exposed specimen at 28°C showing amoebocytic infiltration (arrows a) and degeneration of septae (arrow b); (scale bar = 50 μ). Hematoxylin and eosin

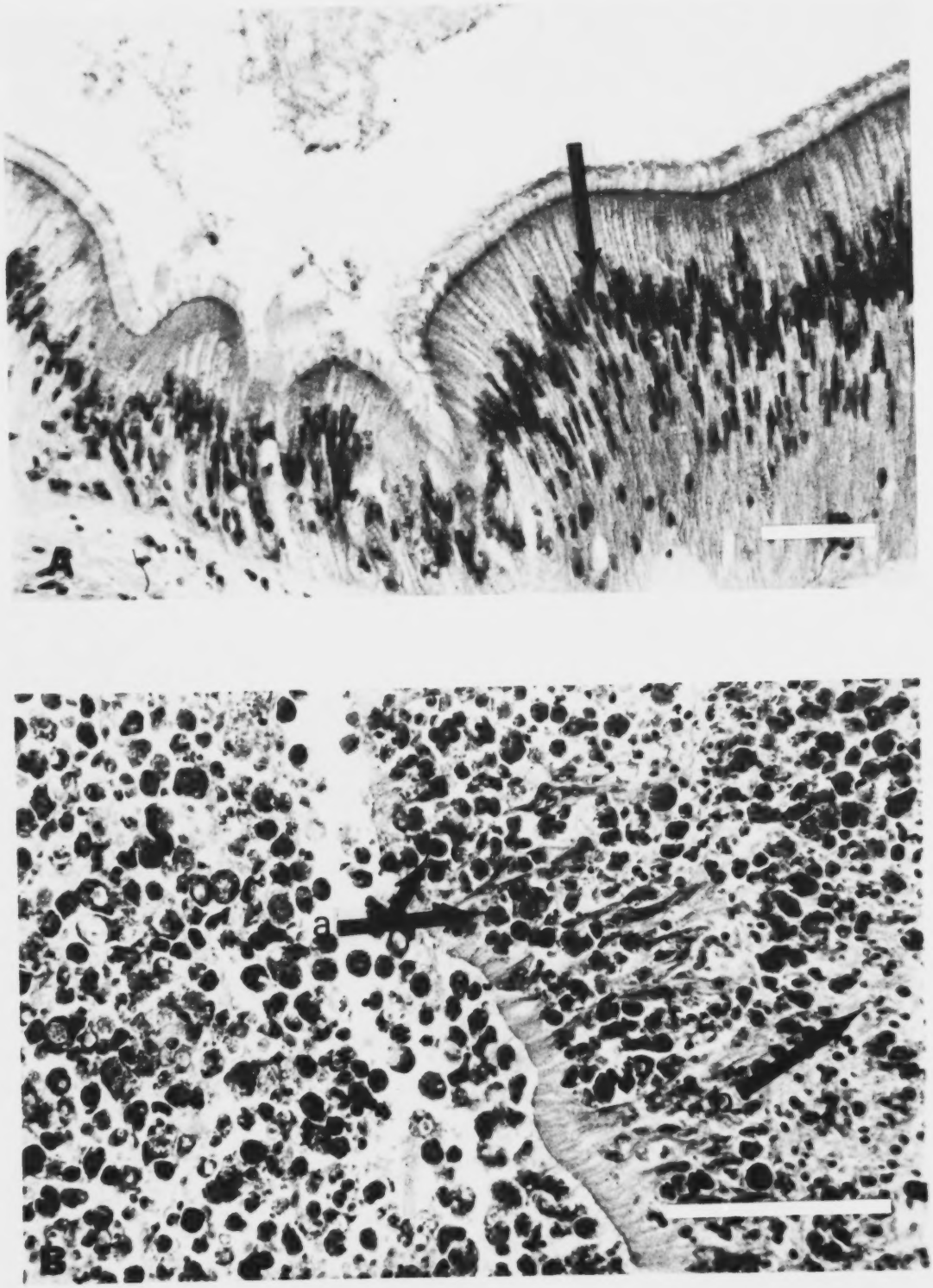

Fig. 8. *Mytilus edulis*. Section of mucosa of stomach of (A) control mussel; note tall filliform columnar epithelium (arrowed) with cilia (scale bar = 100 μ); (B) exposed mussel at 28°C; note infiltration of mucosa by amoebocytes and loss of cilia (arrows a). Also note accumulation of amoebocytes and cellular debris in stomach (arrow b); (scale bar = 50 μ). Hematoxylin and eosin

ture on the filtration rate of *Mytilus edulis* (placed in synonymy with *M. galloprovincialis*). He showed that for Mediterranean mussels the optimum peak of feeding was from 15° to 25°C, but that higher temperatures resulted in almost complete cessation of feeding per unit time. Although the condition of the mussels after 10 days of acclimation at 30°C was not given, it is surprising that they could withstand such high temperatures for such extended length of time. Wallis (1975) studied the thermal tolerance of *M. edulis* from Australia, and reported an ultimate upper incipient lethal temperature of 28.2°C. This appears to be higher than the upper lethal limits reported for *M. edulis* from New England.

One consistent effect of the high temperatures on the mussels was to cause a reduction in feeding at temperatures above 25°C. We believe that part of this decrease in feeding is associated with degeneration of the gill filaments followed by changes in the stomach and intestine. Read (1962) demonstrated that the weight-specific respiration rate of large mussels dropped off markedly at 27°C. The gill damage reported in this paper may be responsible for this change.

Lesions located in the septa of the byssogenous cavity may explain the ease with which mussels detached after being exposed to continuous high temperatures in the laboratory; a response that takes place at the individual and community levels in the heated discharge canal studied here. When temperature stays above 27°C for several days and a colony is pulled off the rocks or pilings in this canal, the mussels literally drop off the thick mat. Moreover, if prolonged high temperatures prevail in the same area, entire mats detach easily and drift away. However, these observations do not explain how dead mussels remained attached to the portion of cyclone fence, which finding led to the undertaking of this research. Wallis (1975) reported that at 25°C byssal thread formation in *Mytilus edulis* from Australia was weak and irregular.

Our results agree with findings and reports of others that the upper lethal temperature of blue mussels is between 27° and 28°C. Nevertheless, we suggest that, because their feeding efficiency is impaired at temperatures above 25°C, they are under stress and cannot tolerate temperatures above this value for extended periods of time. Furthermore, it is concluded that cyclic changes in temperature may have some bearing in extending their viability.

Laboratory experiments, both in the present study and by Read and Cumming (1967), were under continuous selected temperatures, which may account for the quicker response of *Mytilus edulis* to lethal and sublethal temperatures. On the other hand, Pearce (1969) devised an experiment in which he immersed mussels in water of 20°, 25°, and 30°C and back to 25° and 20°C for 6 h at each temperature, after which the cycle was repeated. Mussels thus exposed to this "pseudotide", as he termed it, tolerated the treatment for over 40 days, but when exposed to a continuous 30°C died in 9 to 12 h.

In another study undertaken by G. Pesch (personal communication) the mussels from the Brayton Point study area were shown to be more resistant to heat stress than those obtained from an open-sea station south of Narragansett Bay, Rhode Island. This supports Pearce's (1969) observation that mussels from different ecological zones vary in their response to elevated temperatures. Pearce (1969) showed that *Mytilus edulis* from the intertidal area in Sandy Hook, New Jersey are more tolerant than those from Cape Cod Canal, Massachusetts.

No attempts were made to determine if *Mytilus edulis* recover from lesions developed after subjection to high temperatures.

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